Newborn Screening Quality Assurance Program

PROFICIENCY TESTING

Cystic Fibrosis Quarterly Report

Volume 5, No. 2

May 2006

INTRODUCTION

The Cystic Fibrosis (CF) proficiency testing (PT) report is the quarterly summary of all data reported within the specified data-reporting period for Quarter 2, 2006. The attached tables provide the certification profiles (Immunoreactive Trypsinogen and DNA) for the distributed specimens, the verification of your reported data, the statistical analysis of the quantitative data, and the frequency distributions summary for presumptive clinical (qualitative) assessments. We distribute this PT report to all participants, state laboratory directors, and program colleagues by request.

On April 3, 2006, a panel of five unknown dried-blood-spot (DBS) specimens enriched with predetermined concentrations of IRT was distributed to 16 laboratories in the United States and 51 laboratories in other countries. Nine panels sent to Brazil were returned to CDC because of striking import inspectors.

PARTICIPANTS' RESULTS

We processed data from 53 participants. Laboratories were asked to report IRT results in ng/mL blood. For the statistical summary analysis, we did not include data that were outside the 99% confidence interval. There were 13 outliers for this survey. Results of our evaluation suggest that the endogenous level of IRT was less than 15 ng/mL blood.

Sixteen laboratories reported using Delfia to measure IRT, 26 used AutoDelfia, 2 used MP Biomedicals, 4 used BioRad Quantase, 2 used Bioclone, and the remaining 3 reported using "other." The expected IRT values are based on CDC assayed values. IRT is stable in the dried blood matrix. Table 1 illustrates comparability of the recovery of IRT from each specimen by method.

Presumptive clinical classifications (qualitative assessments) may differ by participant because of specific assessment practices. Participant-specific cutoff values were applied when a non-match occurred between the expected and the reported clinical assessments. Three reported clinical assessments, which were otherwise incorrect, were judged correct by this procedure. Overall, IRT participants reported no false-positive clinical assessments and three false-negative clinical assessments. One laboratory did not report clinical assessments and did not receive an evaluation. Domestic and foreign laboratories reported various cutoffs for IRT. The median and mode cutoffs for domestic participants were 104.1 ng/mL blood and 90 ng/mL blood, respectively. The median and mode cutoffs for foreign participants were 67.4 ng/mL blood and 60 ng/mL blood, respectively.

We distributed DBS specimens containing DNA from Epstein-Barr virus-transformed lymphoblastoid cell lines homozygous or heterozygous for ΔF508 in a sheep or human whole blood matrix (specimens 2682 and 2684). These specimens were enriched with IRT to create proficiency testing materials that expressed both phenotype (elevated IRT) and genotype (ΔF508) for CF.

Participants were asked to confirm specimens that screened IRT positive. Seventeen laboratories reported DNA confirmatory results. Three laboratories reported using PCR amplification of DNA, 4 used Innogenetics Inno-LiPA method, 2 used Abbott Diagnostics oligonucleotide ligation assay, 1 used the Roche Linear Array, 3 used Tm Biosciences Tag-It Cystic Fibrosis kit, 1 used a homemade heteroduplex assay, 1 used PE Applied Biosystems oligonucleotide ligation assay, and 2 used Third Wave Technologies Inplex assay. One laboratory reported an incorrect CF confirmed clinical assessment for specimen 2682, and one laboratory could not report data for specimens 2682 and 2684 because of sample failure. We are continuing to evaluate our methods for preparing these specimens to avoid amplification failures by participants.

The Newborn Screening Quality Assurance Program will ship next quarter's Cystic Fibrosis PT specimens on July 10, 2006.

CDC/APHL

This program is cosponsored by the Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL).

Direct inquiries to:
Centers for Disease Control and Prevention (CDC)
4770 Buford Highway, NE, MS/F43
Atlanta, GA 30341-3724

Phone: 770-488-4023
FAX: 770-488-4255
E-mail: CBell@cdc.gov

Editor: Carol Bell
Production: Connie Singleton
Sarah Brown

CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

NEWBORN SCREENING QUALITY ASSURANCE PROGRAM

CYSTIC FIBROSIS PROFICIENCY TESTING

QUARTER II - MAY 2006

SPECIMEN CERTIFICATION

IRT

CDC ASSAYED LEVELS

Analyte	Specimen 2681	Specimen 2682	Specimen 2683	Specimen 2684	Specimen 2685
Immunoreactive Trypsinogen CDC Mean Assayed Value (ng/mL blood)	20.1 ± 3.1	132.8 ± 6.2	188.9 ± 8.3	149.5 ± 16.4	40.3 ± 2.6

EXPECTED PRESUMPTIVE CLINICAL ASSESSMENTS

Disorder	Specimen 2681	Specimen 2682	Specimen 2683	Specimen 2684	Specimen 2685
Cystic Fibrosis	1	2	2	2	1

1 = within normal limits 2 = outside normal limits 1 or 2 – not evaluated assessment CDC Cutoff: 90 ng/mL blood

DNA

CDC IDENTIFIED GENOTYPES

Analyte	Specimen 2681	Specimen 2682	Specimen 2683	Specimen 2684	Specimen 2685
DNA	Wild Type/ Wild Type	ΔF508/ΔF508	Wild Type/ Wild Type	ΔF508/ΔF508	Wild Type/ Wild Type

EXPECTED DNA CONFIRMED CLINICAL ASSESSMENTS

Analyte	Specimen 2681	Specimen 2682	Specimen 2683	Specimen 2684	Specimen 2685
Cystic Fibrosis	1, 4	2	1	2	1, 4

1 = wild type (normal) 2 = cystic fibrosis positive 3 = cystic fibrosis carrier 4 = not tested

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CYSTIC FIBROSIS - IRT

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OVERALL STATISTICS - IRT

Specimen	N*	Outliers	Mean	UL (95%)	LL (95%)
2681	51	2	19.2	27.4	11
2682	51	2	110.5	145.1	76
2683	50	3	169.5	226.3	112.7
2684	49	4	147.3	185.2	109.4
2685	51	2	33.4	44.2	22.5

* Outliers are not included in N. UL = upper limit LL = lower limit

FREQUENCY DISTRIBUTION OF PARTICIPANTS' CLINICAL ASSESSMENTS

Specimen	Within Normal Limits	Outside Normal Limits
2681	52	0
2682	3	49
2683	0	52
2684	3	49
2685	52	0

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CYSTIC FIBROSIS - DNA

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SUMMARY OF PARTICIPANTS' GENOTYPES

Specimen	Genotype	N
2681	Wild Type/Wild Type Not tested	1 16
2682	ΔF508/ΔF508 ΔF508/WT	15 1
2683	Wild Type/Wild Type Not tested	16 1
2684	ΔF508/ ΔF508	16
2685	Not tested	17

FREQUENCY DISTRIBUTION OF PARTICIPANTS' CLINICAL ASSESSMENTS

Specimen	Wild Type (Normal)	Cystic Fibrosis Positive	Cystic Fibrosis Carrier	Not Tested
2681	1	0	0	16
2682	0	15	1	0
2683	17	0	0	0
2684	0	16	0	0
2685	0	0	0	17

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CYSTIC FIBROSIS - IRT

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IMMUNOREACTIVE TRYPSINOGEN BY METHOD

Table 1. Recovery of IRT (ng/mL blood) by method

Specimen No.	Specimen 2681	Specimen 2682	Specimen 2683	Specimen 2684	Specimen 2685
Expected Value	20.1	132.8	188.9	149.5	40.3
Method (N)					
Delfia (16)	20.5 ± 3.9	113.3 ± 10.9	172.5 ± 14.1	148.5 ± 12.2	33.1 ± 3.8
AutoDelfia (26)	18.9 ± 3.4	114.0 ± 10.6	178.6 ± 13.3	147.3 ± 11.9	34.5 ± 3.8
Bio-Rad (4)	11.4 ± 1.8	89.2 ± 35.2	116.3 ± 21.1	174.4 ± 47.5	26.1 ± 14.5
Other* (7)	19.7 ± 5.8	94.2 ± 33.9	157.8 ± 82.8	130.1 ± 51.5	31.0 ± 10.1

N = Number of observations

*Methods with fewer than 3 users have been grouped into the "Other" category to avoid identifying individual laboratories.